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14. ABSTRACT

The central aim of this project was to utilize "extremophilic" proteins in the fabrication of robust biomaterials. Specific objectives included the development of enabling components that will impart biomaterials with the ability to sense failure and/or repair defects, and the construction of new protein shapes by programmed self-assembly. The biomolecular constituents of these systems are highly thermostable and solvent-resistant chaperone proteins capable of refolding and reactivating denatured proteins under extreme conditions, and a unique filamentous protein recently discovered in the deep-sea hyperthermophile Methanocaldococcus jannaschii (the γ-PFD). The project goals were successfully met through several specific accomplishments, including development of a FRET-based nanosensor for damage-reporting polymeric materials, creation of a partially self-renaturing enzymatic fusion protein, preparation of protein-templated nanowires from the thermostable γ-PFD, and engineering of the γ-PFD scaffold to form nanoscale ovaloids, a new protein architecture.

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Self-Reporting and Detoxifying Materials Based on Extremophilic Proteins

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Final Report, 2010, Contract Number FA9550-07-1-0116

Introduction

This project aims to utilize "extremophilic" proteins in the fabrication of robust biomaterials. Specific objectives include the development of enabling components that will impart biomaterials with the ability to sense failure and/or repair defects, and the construction of new protein shapes by programmed self-assembly. These systems will employ highly thermostable and solvent-resistant chaperone proteins capable of refolding and reactivating denatured proteins under extreme conditions, and a unique filamentous protein recently discovered in the deep-sea hyperthermophile *Methanocaldococcus jannaschii*. Our accomplishments in the specific project categories are summarized below.

Biomechanical Sensors

We have demonstrated the concept of a protein-based nanosensor that is able to report deformation of the embedding polymer matrix. We combined the structural properties of the thermosome (Therm), a chaperonin from the thermophilic organism *Thermoplasma acidophilum*, with the spectral properties of fluorescent proteins in order to generate a protein complex that exhibits fluorescent energy resonance transfer (FRET) and is sensitive to structural deformation. Our concept for a thermosome-based strain sensor is depicted in Figure 1. The Therm-eCFP-eYFP complex was incorporated into polyacrylamide, and the resultant polymeric material was used as a model system to investigate the effect of mechanical deformation of the polymer on the biomechanical sensor. Samples of the polymer were uniaxially strained until they fractured. The area surrounding the fracture face was imaged by multi-channel confocal microscopy (Figure 1). The images clearly show the formation of microcracks due to the applied strain. Self-reporting materials are an intriguing possibility to detect internal damage before occurrence of catastrophic failure of the material. Early detection of impending failure is especially important in cases where the polymer is used as a load-bearing material (e.g., fiber-

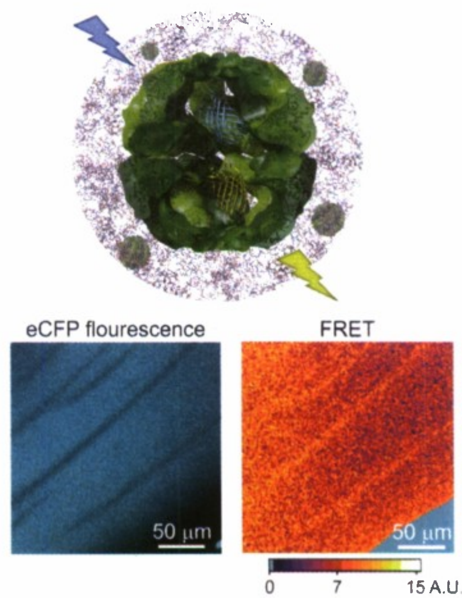

Figure 1. (Top) Thermosome containing a FRET pair of fluorescent proteins (eCFP and eYFP), which can serve as a reporter of structural deformation within polymeric materials. (Bottom) Confocal fluorescence microscopy analysis of strained and fractured polyacrylamide containing the Therm-eCFP-eYFP. Microcracks are clearly evident in both images, as dark lines in the left image and as brighter lines (indicating increased FRET) in the right image.

reinforced polymer composites in automotive and aerospace applications), as polymer adhesive, or as a material in contact with liquids, owing to potential leakage (e.g., tubing, biomedical materials).

Self-renaturing Hybrid Proteins

We have developed a new approach to enzyme immobilization/stabilization in which an enzyme-chaperone chimera is engineered to attach a functional chaperone domain (in this case, a subunit of the recombinant thermosome from *Methanocaldococcus jannaschii*) to the enzyme of interest (the model penicillin amidase, or PGA), creating a single protein with biocatalytic activity and protein refolding capability. This self-renaturing enzyme was further fused to a chitin binding domain to enable simple and effective immobilization. Previous chaperone fusions have been generated for increased expression of aggregation-prone proteins; however, this is the first example of an enzymatically active fusion protein functioning as both a chaperone and an enzyme. Such constructs could serve as components of self-repairing biomaterials in which self-renaturing enzymes facilitate restoration of the material's structure and function.

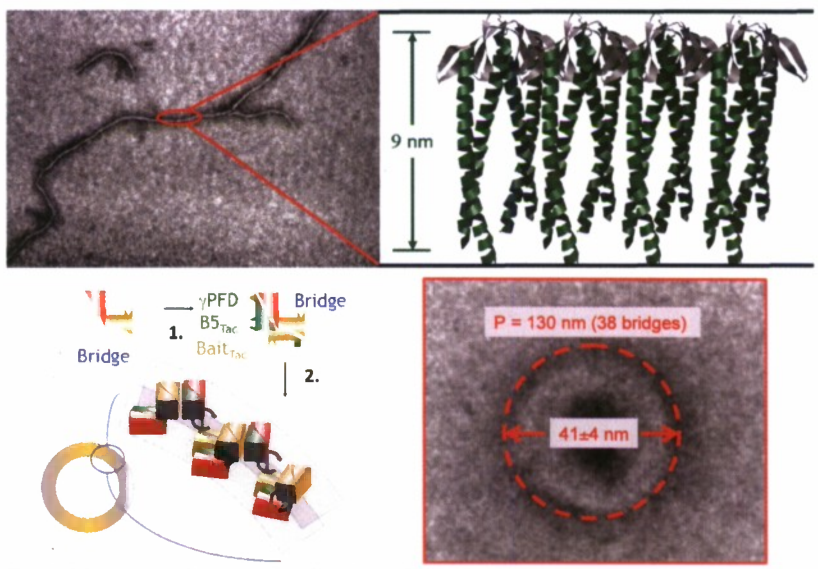

Figure 2. (Top) Electron micrograph of γ-PFD filament (left) and the proposed quaternary structure (right). (Bottom) Proposed self-assembly of engineered γ–PFD subunits ($B5_{Tac}$, in green; and $Bait_{Tac}$, in yellow) and a bivalent bridge structure (red and yellow), which links the adjoined subunits together in a circular structure, or ovaloid. A TEM image of an ovaloid is shown at right. The average diameter of the ovaloids is 41 nm and the perimeter is 130 nm, which corresponds to 38 bridge units per ovaloid.

Programmable Assembly of Biomolecules

We have also achieved a major milestone in protein engineering and biomolecular assembly. Harnessing the potential of proteins as building blocks for the construction of tailor-made scaffolds and templates with specific functions is a longstanding but largely unrealized goal of protein engineering. Peptides and proteins present near endless possibilities as molecular parts for programmed assembly into higher-order structures; however, protein-based structures designed and assembled to date have been based entirely on naturally occurring scaffolds, such as viruses. We have recently discovered a unique filamentous protein in *M. jannaschii* that has enabled us to overcome this limitation. This protein, which we have named the γ-prefoldin (γ-PFD), forms long filaments up to 1 mm in length and of uniform width (8.4±0.4 nm) (Figure 2). These malleable filaments provide an ideal starting point to construct new protein structures, and we have re-engineered the γ-PFD with the overall aim of generating proteins that assemble into 2D and 3D shapes of predictable and controllable dimensions. To our knowledge, such a feat is unprecedented in protein engineering, and signals many new opportunities in protein design and engineering, materials science, and synthetic biology.

In research leading up to our work in programmed self-assembly of 2D protein shapes, we exploited the unique properties of the γ-PFD to mineralize gold, palladium, platinum, and silver along the length of the protein filament to produce conductive wires (in collaboration with Rajesh Naik and co-workers at the Air Force Research Laboratory). γ-PFD filaments serve as excellent templates for the synthesis of metal nanowires of defined length, varied nanoparticle composition, and conductive properties. Beyond single nanoparticle-coated filaments, we also demonstrated the assembly of composite structures in which gold nanoparticles were functionalized with multiple γ-PFD filaments on the gold surface and used to template Pd nanoparticles. Comparatively, these structures exhibited lower resistance (10^1 Ω) than individual coated filaments.

We have also demonstrated the rational design of a capping protein (the **T**hermophilic **E**xtension **R**esistant **M**utant, or TERM) to control the length of the filaments in a modified Flory-Schultz distribution. By combining TERM with the wild-type γ-PFD in varying ratios, the average length and length distribution of the filaments can be varied in a controlled fashion. Finally, we have completely redesigned the modular, full-domain subunits of the γ-PFD (including its TERM variant) to spontaneously form protein ovaloids (Figure 2). The overall strategy used to generate these structures should be generally applicable to protein scaffolds of other shapes and sizes not found in Nature.

Publications Acknowledging AFOSR Support

1. L. M. Bergeron, T. Tokatlian, L. Gomez, and D. S. Clark, "Redirecting the Inactivation Pathway of Penicillin Amidase and Increasing Amoxicillin Production via a Thermophilic Molecular Chaperone," Biotechnol. Bioeng., **102**, 417 (2009).

2. L. M. Bergeron, D. L. Shis, L. Gomez, and D. S. Clark, "Small molecule inhibition of a Group II chaperonin: pinpointing a loop region within the equatorial domain as necessary for protein refolding," Archives of Biochemistry and Biophysics, **481**, 45 (2009).

3. T.A. Whitehead, L.M. Bergeron, and D. S. Clark, "Tying up the Loose Ends: Circular Permutation Decreases the Proteolytic Susceptibility of Recombinant Proteins," Protein Eng. Des. Sel., **22**, 607 (2009).

4. L. M. Bergeron, L. Gomez, T. A. Whitehead, D. S. Clark, "Self-Renaturing Enzymes: Design of an Enzyme-Chaperone Chimera as a New Approach to Enzyme Stability," Biotechnol. Bioeng., **102**, 417 (2009).

5. J. M. Slocik, S. N. Kim, T. A. Whitehead, D. S. Clark, and R. R. Naik, "Biotemplated metal nanowires using hyperthermophilic protein filaments," Small, **5**, 2038 (2009).

6. N. Bruns, K. Pustelny, L. M. Bergeron, T. A. Whitehead, and D. S. Clark, "Mechanical Nanosensor Based on FRET within a Thermosome for Damage-Reporting Polymeric Materials," Angew. Chem. Int. Ed., **48**, 5666 (2009).

7. L.M. Bergeron, C. Lee, and D. S. Clark, "Identification of a Critical Chaperoning Region on an Archaeal Recombinant Thermosome," Biochem. Biophys. Res. Comm., **369**, 7070 (2008).

Summary of Accomplishments

- Developed a FRET-based nanosensor for damage-reporting polymeric materials, which comprised the following steps:

  - Synthesis of a protein-hybrid system where guest proteins such as fluorescent proteins and horseradish-peroxidase are encapsulated into the cavity of the thermosome from *Thermoplasma acidophilum*. This included investigation and optimization of the following strategies:

    - Site-directed mutagenesis of the thermosome and recombinant expression in *E. coli*.
    - Chemical coupling between the thermosome and guest proteins.
    - Stabilization of guest-proteins within the thermosome against thermal inactivation.

  - Encapsulated the FRET-based sensor into polyacrylamide for detection of mechanical stress and formation of micro-cracks.

- Created a partially self-renaturing enzymatic fusion protein to increase the stability, overall activity, and operational lifetime of enzymes in denaturing environments.

- Prepared protein-templated nanowires from thermostable protein filaments (the γ-PFD).

- Engineered the γ-prefoldin scaffold to form nanoscale ovaloid constructs, a new protein architecture.